THE ROLE OF ANXIETY IN A RAT MODEL OF SELF-INJURY

By

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To my parents Dan Ding & Daotian Yuan and my beloved husband Yu Wang

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TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Master Thesis Presented to the Graduate School
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Self-injurious behavior (SIB) is a debilitating characteristic that is highly prevalent in autism, Lesch-Nyhan disease and fragile X syndrome. Although pathological anxiety is also prevalent among these neurodevelopmental disorders, the relationship between anxiety and self-injury has not been adequately characterized. 1) Experiment 1: the open field test (OFT) and elevated plus maze (EPM) were used to assess innate expression of anxiety-related behaviours (ARB) in rats before they were assigned to vehicle-treated (peanut oil, n=6) or pemoline-treated (75 mg/kg/day or 100mg/kg/day, s.c., n=10 per dose) groups. The expression of self-injury was assessed by multiple measures. The low dose of pemoline produced self-injury in 6/10 rats; whereas the high dose did so in all the rats. Correlations between the ARB in the OFT and self-injury were detected, which were absent for the EPM. 2) Experiment 2: independent groups of rats were injected with FG7142 (0, 1, 3, or 10mg/kg, i.p., n=9 per dose) for 5 days, twice daily. Immediately following that, pemoline (75mg/kg, s.c.) was administered to all groups. The expression of self-injury was elevated in the FG7142-treated groups compared to the vehicle. These results suggest that anxiety contributes to vulnerability

for pemoline-induced self-injury. Additional studies of the neurobiological basis of vulnerability for self-injury are currently under investigation.

CHAPTER 1
INTRODUCTION

Self-injury is a devastating characteristic which is prevalent among people with a variety of neurodevelopmental disorders including autism (Richards et al., 2012), fragile X syndrome (Symons et al., 2010), and Lesch-Nyhan syndrome (Schretlen et al., 2007). Intriguingly, anxiety disorders are also found in these and other neurodevelopmental disorders (Hallett et al., 2013; Schretlen et al., 2007; Symons et al., 2010). In addition, the fragile X patients with self-injurious behaviors (SIB) were more likely to have anxiety disorders compared to the fragile X patients who did not self-injure (Symons et al., 2010). However, the potential relationship between anxiety and self-injury has not been clearly established in any of these neurodevelopmental disorders.

Some recent studies in animal models lend support to the notion that anxiety and self-injury might be related. For example, high levels of innate anxiety-related behaviors have been found in SAPAP3 (Welch et al., 2007), Slitrk5 (Shmelcov et al., 2010), and Shank3 (Peça et al., 2011) mutant mice, all of which exhibit stereotypic over-grooming that results in tissue injury. In a rodent model of self-injury, moderate doses of pemoline (an indirect monoamine reuptake inhibitor) can induce SIB in about 50% of rats, demonstrating individual differences in vulnerability for SIB (Kies and Devine, 2004). In addition, dopamine depletion was found in rats repeatedly treated with pemoline (Muehlmann and Devine, 2008). This resembles the dopamine loss in Lesch-Nyhan syndrome (Ernst et al., 1996), although the degree of depletion is far less in the pemoline model, than it is in the genetic syndrome. In another study using the pemoline model (Muehlmann et al., 2011), rats with higher innate locomotor response to the mild stress of a novel environment were more self-injurious than rats with lower locomotor

response, suggesting a possible relation between innate emotional responsiveness and SIB vulnerability. However, the potential contribution of the innate level of anxiety-related behaviors to individual differences in vulnerability for SIB has not been directly tested. In the first of two studies contained herein, both the elevated plus maze test and the open field test were used to prescreen rats to rats in order to obtain a stable measure of their innate anxiety level, before the SIB-inducing pemoline treatment was applied to the same group of rats. Thus, we were able to explore the correlation between innate anxiety-related behaviors and individual differences in vulnerability for pemoline-induced SIB in this study.

Pharmacological research with both humans and non-human primates provides evidence for the potential role of anxiety in SIB, by demonstrating beneficial effects of drugs with anxiolytic properties. One open-label case report (Brahm et al., 2008) indicated that the anxiolytic drug buspirone diminished self-injurious behaviors in an autistic woman, although this should be interpreted with caution because of co-administration of antiepileptic and antipsychotic drugs and staff changes during the treatment. Both buspirone and fluoxetine (an antidepressant drug with anxiolytic actions) attenuated SIB in non-human primates (Fontenot et al., 2005; Fontenot et al., 2009), whereas diazepam produced inconsistent effects on pemoline-induced SIB in rats (Mueller and Nyhan, 1982). However, the impact of anxiogenic agent on SIB has only been tested in one study, using non-human primates, which produced mixed results. (Major et al., 2009). FG7142 (a partial inverse agonist of GABAA receptors) increases the expression of anxiety-related behaviors in the Vogel conflict test (Stephens et al., 1987), elevated plus maze test (Atack et al., 2005; Dawson et al.,

2006), elevated T maze test (Bueno et al., 2005; Sena et al., 2003) and social interaction test (Hackler et al., 2007). Also, it increases expression of both c-fos protein and mRNA in brain regions involved in anxiety-related-behaviors, which include amygdala, hypothalamus, bed nucleus of stria terminalis, etc. (for review, see Evans and Lowery, 2007). In the second study contained herein, we tested the effects of FG7142 on frequency and severity of self-injury induced by pemoline in rats.

CHAPTER 2
METHODS

Animals and Drugs

Animals

Sixty two male Wistar rats (Charles River Laboratories, Raleigh, NC) weighing 176~200g were used in this study, with 26 of them used in experiment 1 and 36 rats used in experiment 2. All the rats were paired-housed in standard polycarbonate cages (43 cm×21.5 cm×25.5 cm) under a 12 hr:12 hr light/dark schedule (lights on at 7:00 am) with controlled temperate and humidity. Standard rat chow (Lab Diet 5001) and tap water were available ad libitum. Each rat was acclimated to the housing conditions for a week prior to the start of the experiment. All the experimental procedures were pre-approved by the Institutional Animal Care and Use Committee at the University of Florida, and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

Drugs

Pemoline (Spectrum Chemicals, New Brunswick, New Jersey) was suspended in peanut oil at 50 mg/mL. FG7142 (Sigma-Aldrich Inc., St. Louis, MO) was suspended in 1.0% (w/v) carboxymethylcellulose (CMC) at concentrations of 0, 1, 3, or 10 mg/mL.

Experimental Procedures

Experiment 1

Behavioral pre-screening

Three behavioral tests: elevated plus maze (EPM), open field test (OFT) and circular corridor test (CCT) were conducted on experimental days 1, 3, and 5, respectively, between 0800 and 1300.

Elevated plus maze (EPM). The EPM apparatus was constructed with black acrylic, with four 45cm×12cm arms extended from one 12cm×12cm central platform. Two opposing closed arms were surrounded by 45cm high walls, and the two open arms had 0.6cm high ledges. The height of the maze was 90cm above the floor. Illumination (provided by a light bulb above the central platform of the maze) was 46-60 lux in the open arms, 19-30 lux in the closed arms and 43-45 in the central platform. A video camera suspended above the maze was used to record the rats' activities during tests.

At the beginning of the test, one individual rat was placed on the central platform facing one of the closed arms. Then it was allowed to explore the maze during the 5-minute test session. All activities during the period were digitally-recorded and assessed using "Noldus Observer" software (Noldus Information Technology) by the experimenter. Latency to enter the open arms was defined as the interval between the beginning of the session and the first time when the rat placed all four paws into any one of the two open arms. The time spent in the closed arms was defined as the total duration of all visits to the closed arms during the test session, which began when the rat's four paws were into one of the closed arms, and ended when the rat's four paws were into one of the open arms. The maze was cleaned with 4% bleach and dried between each individual test session.

Open field test (OFT). The OFT apparatus was a 90cm×90cm black acrylic arena (open area) connected to a 20cm×30cm start box (closed area). The open area and start box were separated by a 20cm×20cm acrylic door. The door was connected to a rope that allowed it to be opened from outside the test room. The walls of the

apparatus were 60cm high, and there was an open top. The illumination was 12-15 lux in the closed area and 28-29 lux in the open area. A digital camera mounted above the apparatus was used to record the rats' behaviors during the test.

Each rat was individually placed into the start box, and the door separating the open area and the start box was kept closed for 1 min. The door was then opened remotely, and a 5-minute test session started. Behaviors during the test session were assessed using "Noldus Observer" software by the experimenter. Latency to enter the open area was measured as the interval from the moment when the door was opened to the first time when the rat placed all four paws into the open area. Time spent in the closed area was measured as the total time of all visits to the closed area during the test session, which started/ended when the rat placed all four paws into the closed/open area. The apparatus was cleaned with 4% bleach and dried between each individual test session.

Circular corridor test (CCT). The CCT apparatus was a plastic bucket with another smaller bucket set within it. The center of the smaller bucket was aligned with that of the larger one, forming a 7cm wide circular corridor between the walls of the two buckets. The outer diameter of the circular corridor was 44cm. The illumination during tests was 8-12 lux within the circular corridor, provided by a lamp placed on the floor outside the larger bucket. At the beginning of the test, each rat was individually placed into the circular corridor and then the video camera above the apparatus was used to record the rat's activities during the 1-hour test session. Behavioral stress responsiveness to the mild stress of this novel environment was scored as the counts of "line-crossings" within the circular corridor. A transparent sheet of acetate with two lines

perpendicular to each other, was placed over the video image on the computer monitor, to divide the image of the circular corridor into 4 quadrants. A “line-crossing” was counted each time the rat’s snout “crossed” a line, any individual line was not counted again until the rat crossed another different line. The circular corridor was cleaned with 4% bleach, dried and then filled with fresh standard bedding between each individual test session. The rats were transferred to single-housing in identical polycarbonate cages upon completion of the CCT.

Pemoline treatment

Pemoline treatment was applied between Day 13 to Day 22. Independent groups of rats were assigned to drug treatments in a balanced manner based on their counts of line-crossings in the CCT. The rats were injected once daily with 0, 75, or 100 mg/kg, s.c, at 0900-1000 during the treatment. The pemoline injections were administered either at the nape of the neck, or the dorsal flank near the right hindlimb. Each group had 10 rats except the control group that had 6 rats.

Assays of SIB and ancillary behaviors

At 0900 and 1700 of each day during the pemoline treatment, each rat was inspected to examine physical evidence of injury. Each rat was held before a digital camera to record any injury sites (denuded skin, erythema, edema, and open lesions). Tissue injury scores were assigned to each rat according to a 0-4 rating scale (See table 1). Injury sites were digitally recorded and still images were captured. The size of each injury site was measured using MCID image analysis software (Imaging Research Inc., St. Catharines, ON, Canada). Average tissue injury scores and average injury size were calculated across days for each rat.

Digital recordings of the rats' behaviors were recorded using digital cameras for 4 time samples each day between 0600-0615, 1200-1215, 1800-1815 and 2400-0015 for 5 minutes each time, recording the rats' behaviors from their home cages throughout the pemoline treatment (total 200 minutes for each rat). Self-injurious oral contact and other ancillary behaviors (stereotypy, grooming, and rearing) were assessed by the experimenter using "Noldus Observer" software. Self-injurious oral contact was defined as all oral contact that stayed fixed on any particular body site for 2 seconds or longer. For each of the 5-minute time samples, the total duration summed from all bouts of self-injurious oral contact was quantified. Average duration of oral contact was calculated across all 40 time samples for each rat. Grooming was defined as whenever a rat made oral contact along its torso or limbs, staying fixed at any particular site for less than 2 seconds. Stereotypy was defined as whenever a rat continuously engaged in any of the following behaviors for over 3 seconds: cage licking, head bobbing and/or digging/burrowing/sniffing through the bedding. Since the expression of the specific forms of stereotypy was different for each individual rat, the stereotypy scores are reported as aggregate scores that compile the total duration of all these stereotyped behaviors. Rearing was defined as whenever a rat raised both of its forepaws off the bedding for at least 3 seconds. The daily average duration of each of the ancillary behaviors was calculated across the four time samples from the same day for each rat. For each of the ancillary behaviors, average duration was calculated across all 40 time samples for each rat.

Termination

The rats were terminated 24 hrs after the final pemoline injection on the morning of the 23th experimental day, except those that were euthanized early due to severe

tissue damage (i.e. reached score 4 according to the 0-4 rating scale: Table. 1 (Kies and Devine, 2004)).

Statistical analyses

One-way analyses of variance (ANOVAs) were used to compare each measure of the behavioral tests (EPM, OFT and CCT) between doses to assess balanced group assignment. Differences in each of the measures of self-injury and ancillary behaviors (grooming, stereotypy and rearing) between pemoline dose groups were analyzed using repeated measures analyses of variance (RM-ANOVAs). Significant effects ($p<0.05$) were further analyzed with the Bonferroni post-tests. The relationships between two measures of self-injury (daily average duration of oral contact and injury size and tissue injury score) and the measures of the anxiety-related behaviors in the EPM and OFT (latency to enter the open arms, time spent in the closed arms, latency to enter the open area, time spent in the closed area) were each evaluated with Pearson's correlation analyses. Also, the relationships between the tissue injury score and the measures of the anxiety-related behaviors in the EPM and OFT were each evaluated with Spearman's correlation analyses. Results were treated as significant if the P-value (1-tailed) was less than 0.05. Similarly, the relationships between measures of stress responsiveness in the CCT (novelty-induced locomotor counts) and any measure of self-injury were also evaluated by Pearson's (or Spearman's for tissue injury score) correlation analyses. Again, the relationships between daily average duration of the ancillary behaviors (grooming, stereotypy and rearing) and the behavioral measures in the EPM, OFT and CCT (latency to enter the open arms, time spent in the closed arms, latency to enter the open area, time spent in the closed area, and novelty-induced

locomotor counts) were each evaluated with Pearson's correlation analyses. Results were treated as significant if the P-value (1-tailed) was less than 0.05.

Experiment 2

Open field test (OFT)

On day 1 of Experiment 2 (i.e. after 7-days acclimation), the OFT was conducted according to the procedure described for Experiment 1.

Drug treatments

The drug treatments were administered during experimental days 3-7. Upon initiation of the drug treatments, the rats were singly-housed in identical polycarbonate cages in order to ascertain that all injuries were self-inflicted. The rats were assigned to different doses of the FG7142 treatment in a balanced manner based on their performance in the OFT (the time spent in the closed area). These independent groups of rats were injected with FG7142 (0, 1, 3, or 10mg/kg, i.p., n=9 per group) twice daily at 0800-0845 and 1700-1745 throughout the treatment. Immediately following the FG7142 injection each morning, pemoline (75mg/kg, s.c) was administered to all groups. The pemoline injections were administered either at the nape of the neck, or the dorsal flank near the right hindlimb.

Assays of SIB and other ancillary behaviors

Rats were inspected in the afternoon the day before the start of the drug treatments. Throughout the treatments, daily inspections were conducted at 1700-1745 for each rat before FG7142 was administered, according to the procedures described for Experiment 1. The tissue injury scores were assigned using the previously described tissue injury rating scale (Table. 1-1). Time sample recordings of the rats' behaviors were collected by digital cameras 4 times a day for 5 minutes each time between 0115-

0200, 0845-0930, 1315-1400, and 1745-1830, recording the rats' behaviors from their home cages throughout the treatments. Self-injurious oral contact and ancillary behaviors (stereotypy, grooming, and rearing) were assessed using "Noldus Observer" software by the experimenter, according to the procedures described for Experiment 1; except that the self-injurious oral contact was counted only when the oral contact stayed fixed at any particular body site for 5 seconds or longer, and grooming was defined as whenever a rat made oral contact along its torso or limbs while staying fixed at any particular site less than 2 seconds.

Termination

The rats were terminated on the morning of day 8 except those that were euthanized early due to severe tissue damage (i.e. reached score 4 according to the self-injury rating scale: Table. 1-1).

Statistical analyses

One-way analyses of variance (ANOVAs) were used to compare OFT measures (latency to enter the open area and time spent in the closed area) between doses to assess balanced group assignment. Differences in each of the measures of self-injury and ancillary behaviors (grooming, stereotypy and rearing) between FG7142 dose groups were analyzed using repeated measures analyses of variance (RM-ANOVAs). Significant effects ($p<0.05$) were further analyzed with the Bonferroni post-tests.

Table 1-1. Rating scale of tissue injury

Score	Severity	Description
0	No SIB	No tissue damage
1	Very Mild SIB	Slight edema, pink moist skin, involves small area
2	Mild SIB	Moderate edema, slight erythema, slightly denuded skin, involves medium area, and/or involves multiple sites
3	Moderate SIB	Substantial edema and erythema, large area, substantially denuded skin, and/or involves multiple sites
4	Severe SIB	Clear/open lesions, and/or amputation of digit, requires immediate euthanasia

CHAPTER 3
RESULTS

Experiment 1

Evaluation of Balanced Group Assignments

Since the rats were assigned to the groups in a counterbalanced manner based upon the locomotor scores in the CCT, one-way ANOVAs were used to evaluate how evenly the groups were distributed in scores on CCT and the other two pre-screening tests. For the counts of line crossings in the CCT, the one-way ANOVA revealed no significant between groups differences ($F_{2, 23}$= 0.2379, P>0.1; Fig. 3-1A) among different pemoline doses. For the measures in the EPM, the one-way ANOVAs revealed no significant between-groups differences for the time spent in the closed arms ($F_{2, 23}$=1.856, P>0.1; Fig. 3-1B) or the latency to enter the open arms ($F_{2, 23}$=1.081, P>0.1; Fig. 3-1C). For the measures in the OFT, the one-way ANOVAs revealed no significant between-groups differences for the time in the closed area ($F_{2, 23}$=2.644, P>0.05: Fig. 1D) or the latency to enter the open area (F 2, 23= 1.209, P>0.1; Fig. 3-1E).

Dose Effects of the Pemoline Treatment on Self-Injury

The measures of pemoline-induced self-injury (the duration of oral contact, the injury size and the tissue injury score) were higher in the two pemoline-treated groups than in the vehicle-treated group, and were higher at the high dose (pemoline 100mg/kg) compared with the low dose (pemoline 75 mg/kg). For the duration of oral contact (Fig. 3-2A), the 2-way RM-ANOVAs revealed a significant main effect of dose ($F_{2, 198}$=10.31, p<0.001), a significant main effect of time ($F_{9, 198}$=4.55, p<0.0001), and a significant dose x time interaction ($F_{18, 198}$=2.04, p<0.01). For the injury size (Fig. 3-2B), the 2-way RM-ANOVAs revealed a significant main effect of dose ($F_{2, 230}$=15.41,

$p<0.0001$), a significant main effect of time ($F_{10, 230}=14.97$, $p<0.0001$), and a significant dose x time interaction ($F_{20, 230}=8.80$, $p<0.0001$). For the tissue injury score (Fig. 3-2C), 2-way RM-ANOVAs revealed a significant main effect of dose ($F_{2, 230}=24.58$, $p<0.0001$), a significant main effect of time ($F_{10, 230}=26.92$, $p<0.0001$), and a significant dose x time interaction ($F_{20,230}=13.80$, $p<0.0001$). The percentage of rats with tissue injury across each day of the treatment is shown in Fig. 3-2D.

Between-Group Differences in Ancillary Behaviors

For the duration of grooming (Fig. 3-3A), the 2-way RM-ANOVAs revealed a significant main effect of dose ($F_{2, 198}=7.53$, $p<0.01$), whereas the effect of time ($F_{9, 198}=0.87$, $p>0.1$), and the dose x time interaction ($F_{18, 198}=0.77$, $p>0.1$) were not significant. For the duration of stereotypy (Fig. 3-3B), the 2-way RM-ANOVAs revealed a significant main effect of dose ($F_{2, 198}=12.72$, $p<0.001$), a significant main effect of time ($F_{9, 198}=12.71$, $p<0.0001$), and a significant dose x time interaction ($F_{18, 198}=3.60$, $p<0.0001$). For the duration of rearing (Fig. 3-3C), the 2-way RM-ANOVAs revealed a significant main effect of dose ($F_{2, 198}=3.84$, $p<0.05$), a significant main effect of time ($F_{9, 198}=8.84$, $p<0.0001$), and a significant dose x time interaction ($F_{18, 198}=2.27$, $p<0.005$).

Correlations between the Average Measures of Self-Injury and the Measures of Innate Anxiety-Related Behaviors

Correlations between the average measures of self-injury and the OFT measures

At the low dose (pemoline 75 mg/kg) group, each pair of the Pearson correlations between self-injury measures (average oral contact duration and average injury size) and the OFT measures (time spent in the closed area and latency to enter the open area) was significant. The Spearman correlation between the average tissue injury score and the OFT measures was also significant for the low dose group (see Fig. 3-4

and Table 3-2). At the high dose (pemoline 100mg/kg) group, the correlation was significant between the average injury size and the time spent in the closed area (see Fig 3-5 and Table 3-2).

Correlations between the average measures of self-injury and the EPM measures

There were no significant correlations between measures of anxiety-related behavior on the EPM and measures of self-injury for the low dose (see Fig 3-6 and Table 3-2). For the high dose (pemoline 100mg/kg) groups, the Pearson correlations between the average duration of oral contact and the time spent in the closed arms were significant. Also at the high dose, the Pearson correlation between the average injury size and time spent in the closed arms was significant (see Fig 3-7 and Table 3-2).

Correlations between the average measures of self-injury and the locomotion response to mild stress in the CCT

The Pearson correlation between the average injury size and the counts of line crossings in the CCT was significant at the low dose (pemoline 75mg/kg; see Fig 3-8, Table 3-2). And there was no significant correlation detected for the high dose (see Fig 3-9, Table 3-2).

Correlations between the Average Duration of Ancillary Behaviors and the Measures of Innate Anxiety-Related Behaviors

The Pearson correlations between the average duration of ancillary behaviors (grooming, stereotypy and rearing) and the measures in the EPM, the OFT and the CCT (time spent in the closed arms, latency to enter the open arms, time spent in the closed area, latency to enter the open area, and the counts of line crossing) are illustrated in Table 3. The Pearson correlation between the average duration of grooming and the time spent in the closed arms in the EPM was significant for the vehicle-treated group

(see Table 3-3). Also, the correlation between the average duration of stereotypy and the time spent in the closed arms in the EPM was significant at the pemoline dose of 100 mg/kg (see Table 3-3).

Experiment 2

Evaluation of Balanced Group Assignments

Since the group assignments were counterbalanced on the basis of time spent in the closed area of the OFT in this experiment, the 1-way ANOVAs confirmed that there were no significant between-groups differences in the time spent in the closed area ($F_{3, 32}$=0.0071, P>0.1). Likewise, there were no significant between-groups differences in the latency to enter the open area ($F_{3, 32}$ =0.3240, P>0.1). (See Fig 3-10.)

FG7142-Induced Elevation in Expression of Self-Injury

Nine rats were euthanized before completion of the treatments due to severe tissue damage (i.e. reached score 4 according to the 0-4 rating scale). (See Table 3-4). Thus, the missing data of the self-injury measures for the 9 rats were replaced by the corresponding data of the last day before the euthanization from the euthanization day to the end of the experiment.

The FG7142-treated groups had higher scores on the duration of oral contact compared with the vehicle-treated group (Fig. 3-11A). The 2-way RM-ANOVAs revealed a significant main effect of dose ($F_{3, 128}$=3.26, p<0.05) and a significant main effect of time ($F_{4, 128}$=20.42, p<0.0001) on the duration of oral contact. The dose x time interaction was not significant ($F_{12, 128}$=1.17, p>0.05). For the tissue injury score (Fig. 3-11B), two-way RM-ANOVAs revealed no significant effect of dose ($F_{3, 160}$=2.06, p>0. 1) on the tissue injury score, but there were significant effects of time ($F_{5, 160}$=97.44, p<0.0001) and of the dose x time interaction ($F_{15, 160}$=1.96, p<0.05). Also, the

percentage of rats with tissue injury across each day of the treatments was demonstrated in Fig. 3-11C.

Between-Group Differences in Ancillary Behaviors

The 2-way RM-ANOVAs revealed a significant effect of time ($F_{4, 128}$=4.01, p<0.01) on the duration of grooming (Fig. 3-12A), but there were no significant effects of dose ($F_{3, 128}$=0.84, p>0.1) or the dose x time interaction ($F_{12, 128}$=0.32, p>0.1). The 2-way RM-ANOVAs revealed a significant effect of dose ($F_{3, 128}$=3.27, p<0.05) and time ($F_{4, 128}$=19.10, p<0.0001) and on the duration of stereotypy (Fig. 3-12B), but there were no significant effects of the dose x time interaction ($F_{12, 128}$=0.93, p>0.1). The 2-way RM-ANOVAs revealed a significant effect of time ($F_{4, 128}$=30.10, p<0.0001) on the duration of rearing (Fig. 3-12C), but there was no significant effect of dose ($F_{3, 128}$=0.69, p>0.1) or the dose x time interaction ($F_{12, 128}$=1.44, p>0.1).

Table 3-1. The correlations between the average measures of self-injury and the measures of innate anxiety-related behaviors. The Pearson's r was calculated for each pair of the correlations between the measures in OFT, EPM or CCT and the average duration of oral contact or the average injury size. The Spearson's rho was calculated for each pair of the correlations between the measures in OFT, EPM or CCT and the average tissue injury score. All significant correlations are depicted in bold font. The significance of each correlation is depicted as *p<0.05 and **p<0.01 (1-tailed). One rat in the low dose group (pemoline 75mg/kg) was excluded from analyses with the duration of oral contact due to data loss, which left 9 rats in the group.

		OFT				EPM				CCT	
		time closed area		latency open area		time closed arm		latency open arm		line crossing	
		p-value	*r* or rho	p-value	*r* or rho	p-value	*r* or rho	p-value	*r* or rho	p-value	*r* or rho
75 mg/kg (n=9 or 10)	oral contact	*0.0066* **	0.7796	*0.0028* **	0.8309	0.4624	0.03696	0.43	-0.06897	0.104	-0.4643
	injury size	*0.0242* *	0.6355	*0.0163* **	0.6739	0.4749	-0.02299	0.305	-0.1845	*0.0235* *	-0.6382
	tissue injury score	*0.0195* *	0.6667	*0.0441* *	0.5749	0.4191	0.07927	0.5	-0.006098	0.2801	-0.2141
100 mg/kg (n=10)	oral contact	0.1913	-0.3105	0.4697	-0.02768	*0.0429* *	-0.5695	0.2865	-0.2034	0.0839	-0.4725
	injury size	*0.0245* *	-0.634	0.3476	-0.1421	*0.006* **	-0.7522	0.1678	-0.3406	0.4822	-0.01626
	tissue injury score	0.2801	-0.2067	0.4191	0.07295	0.0893	-0.462	0.2135	0.2857	0.2801	-0.2128

Table 3-2. The correlations between the average duration of the ancillary behaviors and the measures of innate anxiety-related behaviors. The Pearson's r was calculated for each pair of correlations between the measures in OFT, EPM or CCT and the average duration the ancillary behaviors. The significance of each correlation was depicted as *p<0.05 (1-tailed). One rat in the low dose group (pemoline 75mg/kg) was excluded from analyses with the duration of ancillary behaviors due to data loss caused by a computer error, which left 9 rats in the group.

		OFT				EPM				CCT	
		time closed area		latency open area		time closed arms		latency open arms		line crossing	
		p-value	r	p-value	r	p-value	r	p-value	r	p-value	r
0 mg/kg (n=6)	grooming	0.4674	0.04344	0.0718	-0.6722	*0.0172* *	-0.8447	0.4255	-0.09961	0.2213	0.3915
	stereotypy	0.0945	-0.6202	0.3025	-0.2698	0.409	-0.122	0.4143	-0.1148	0.2051	-0.4174
	rearing	0.2389	0.3643	0.292	-0.2851	0.0764	-0.6612	0.2426	-0.3586	0.0624	0.6957
75 mg/kg (n=9)	grooming	0.2382	0.2735	0.3229	0.1786	0.4494	-0.04976	0.2222	-0.2928	0.4886	-0.01115
	stereotypy	0.3474	-0.1527	0.477	-0.02254	0.4871	-0.01263	0.2178	-0.2983	0.1104	0.4529
	rearing	0.2054	-0.3139	0.2437	-0.267	0.1951	0.3271	0.2699	0.2367	0.1028	0.4664
100 mg/kg (n=10)	grooming	0.4345	0.06012	0.4136	-0.07949	0.2221	0.2737	0.2927	-0.197	0.3664	0.124
	stereotypy	0.2232	0.2724	0.4602	0.0364	*0.0127* *	0.6958	0.1301	0.3937	0.1046	0.4348
	rearing	0.4651	0.03196	0.382	0.1092	0.302	0.1875	0.1129	-0.4208	0.1734	0.3333

Table 3-3. Euthanized rats in experiment 2

	total # of rats euthanized	euthanization time (# of rats euthanized at the time)
0 mg/kg FG7142	1	day 3 (1)
1 mg/kg FG7142	3	day 3 (1), day 4 (1), day 5 (1)
3 mg/kg FG7142	4	day 3 (2), day 4 (1), day 5 (1)
10 mg/kg FG7142	1	day 4 (1)

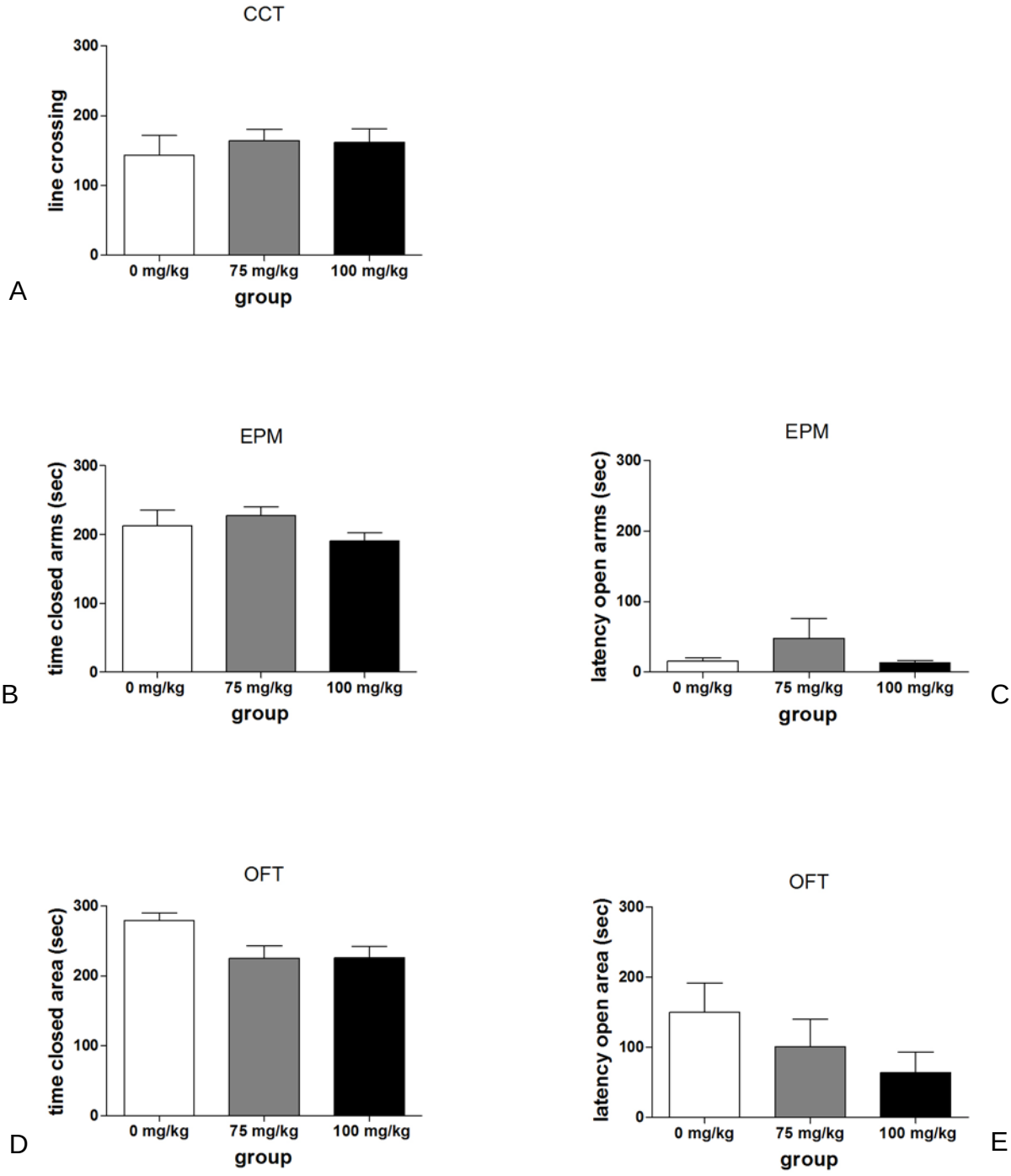


Figure 3-1. Evaluation of balanced group assignments for Experiment 1. The balance of the group assignment was confirmed as the means of A) the counts of line crossings in the CCT. This balancing of assignment based on the CCT scores also produced groups that were balanced on the B) time spent in the closed arms in the EPM, C) latency to enter the open arms in the EPM, D) time spent in the closed area in the OFT, E) latency to enter the open area in the OFT (n=10 for the pemoline-treated groups, n=6 for the vehicle group). Values expressed are group means ± SEM.

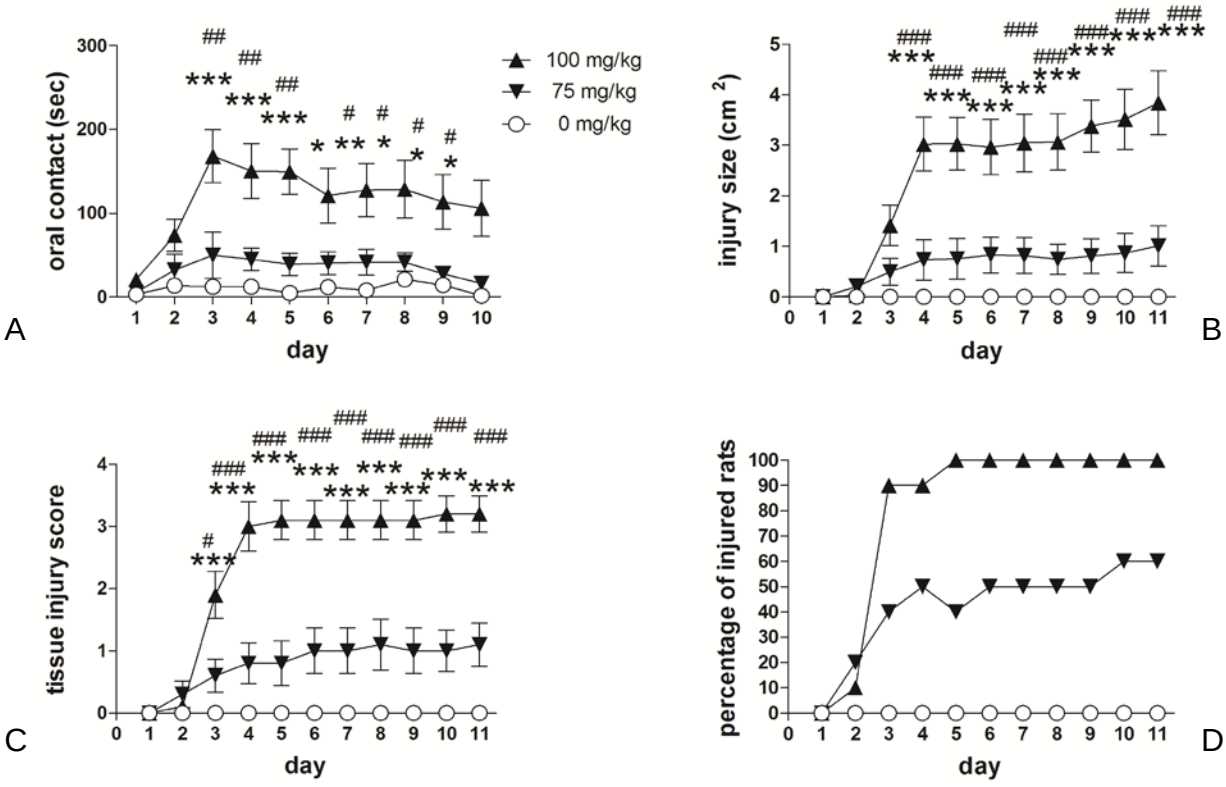


Figure 3-2. The dose effects of the pemoline treatment. A) Duration of oral contact, B) injury size and C) tissue injury score, D) percentage of rats with tissue injury. Significant differences between the high dose (pemoline 100mg/kg), the low dose (pemoline 75 mg/kg), and the vehicle groups are presented as follows: *P<0.05, **P<0.01, ***P<0.001 for comparisons between the pemoline dose at 100 mg/kg and the vehicle; # P<0.05, ## P<0.01, ### P<0.001 for comparisons between the pemoline dose at 100 mg/kg and 75mg/kg. As four rats reached the tissue injury score 4 in the high dose group were euthanized at the morning of day 4, the missing data points of oral contact, injury size and tissue injury scores from day 4 to the end of the experiment were replaced by the corresponding data of day 3 for those rats. n=10, for the pemoline-treated groups; n=6, for the vehicle group. Values expressed are group means ± SEM.

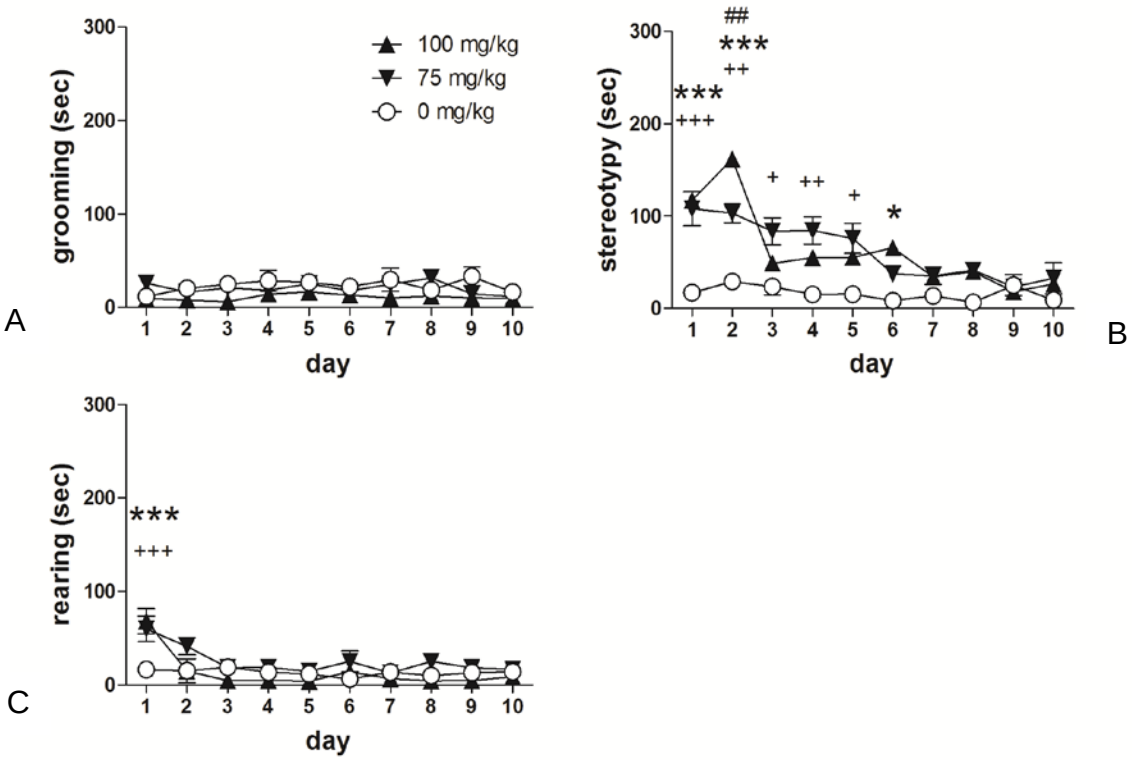
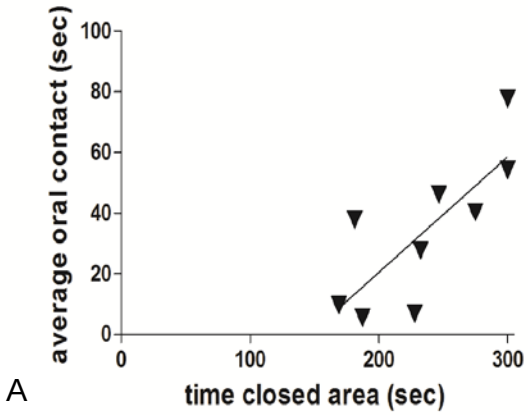


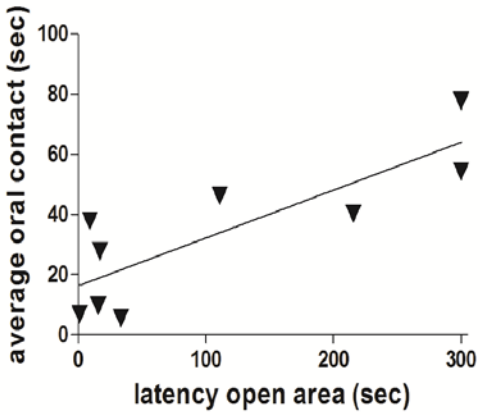
Figure 3-3. Between-group differences in duration of ancillary behaviors in experiment 1. A) Duration of grooming, B) duration of steretypy; C) duration of rearing. Significant differences among the high dose (pemoline 100mg/kg), the low dose (pemoline 75 mg/kg) and the vehicle groups are presented as follows: $^{+}$P<0.05 , $^{++}$P<0.01, $^{+++}$P<0.001 for comparisons between the pemoline dose at 75 mg/kg and vehicle; *P<0.05, ***P<0.001 for comparisons between the pemoline dose at 100 mg/kg and vehicle; # P<0.05 for comparisons between the pemoline dose at 100 mg/kg and 75mg/kg. As four rats reached the tissue injury score 4 in the high dose group were euthanized at the morning of day 4, the missing data points of the duration of the ancillary behaviors from day 4 to the end of the experiment were replaced by the corresponding data of day 3 for those rats. n=10 for the pemoline-treated groups; n=6 for the vehicle group.

Figure 3-4. The correlations between self-injury and innate anxiety-related behaviors in the OFT at the pemoline dose of 75 mg/kg. A) The significant positive correlation ($p<0.01$, $r=0.7796$) between the average duration of oral contact and the time spent in the closed area. B) The significant positive correlation ($p<0.01$, $r=0.8309$) between the average duration of oral contact and the latency to enter the open area. C) The significant positive correlation ($p<0.05$, $r=0.6355$) between the average injury size and the time spent in the closed area. D) The significant positive correlation ($p<0.05$, $r=0.6739$) between the average injury size and the latency to enter the open area. E) The significant positive correlation ($p<0.05$, $r= 0.6667$) between the average tissue injury score and the time spent in the closed area. F) The significant positive correlation ($p<0.05$, $r=0.5749$) between the average tissue injury score and the latency to enter the open area. One rat in the low dose group (pemoline 75mg/kg) was excluded from analyses with the duration of oral contact due to data loss, which left 9 rats in the group.

75 mg/kg pemoline



A



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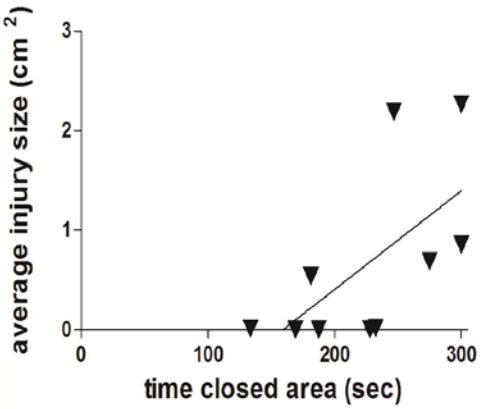

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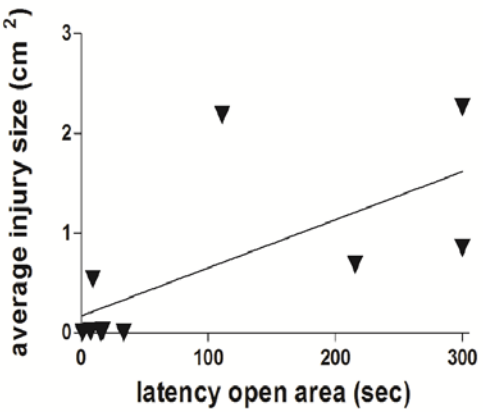

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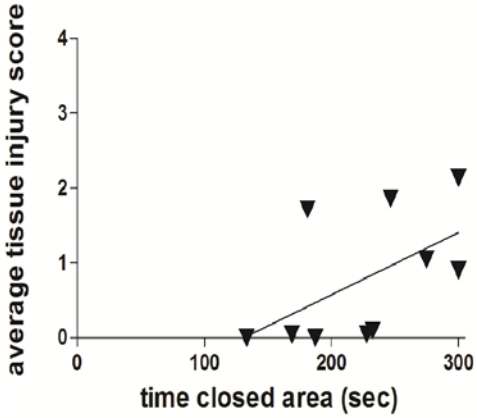

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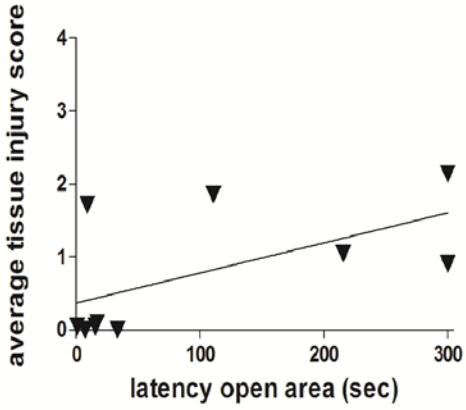
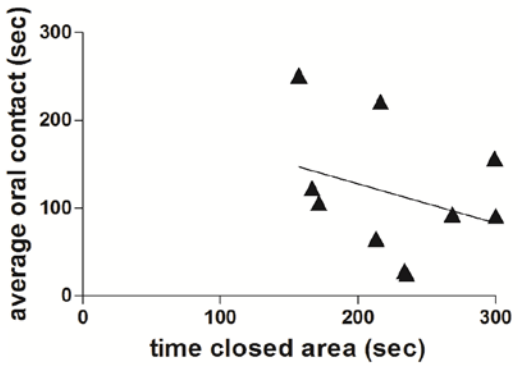


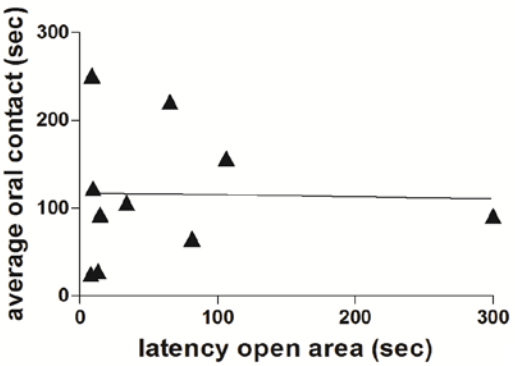
F

Figure 3-5. The correlations between self-injury and innate anxiety-related behaviors in the OFT at the pemoline dose of 100 mg/kg. A) There was no significant correlation ($p>0.05$, r=-0.3105) between the average duration of oral contact and the time spent in the closed area. B) There was no significant correlation ($p>0.05$, r=-0.02768) between the average duration of oral contact and the latency to enter the open area. C) There was a significant negtive correlation ($p<0.05$, r=-0.634) between the average injury size and the time spent in the closed area. D) There was no significant correlation ($p>0.05$, r=-0.1421) between the average injury size and the latency to enter the open area. E) There was no significant correlation ($p>0.05$, r= -0.2067) between the average tissue injury score and the time spent in the closed area. F) The was no significant correlation ($p>0.05$, r=0.07295) between the average tissue injury score and the latency to enter the open area.

100 mg/kg pemoline



A



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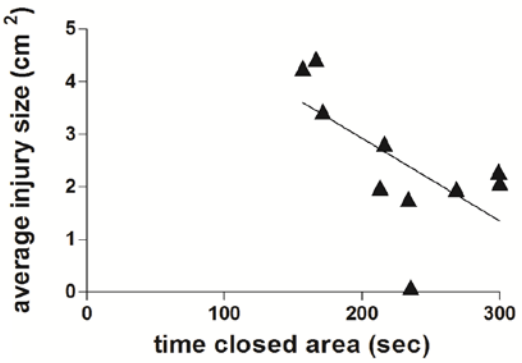
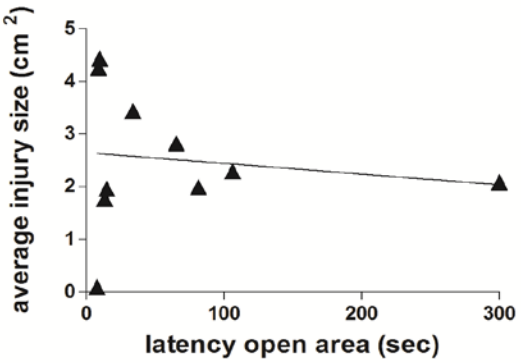


C



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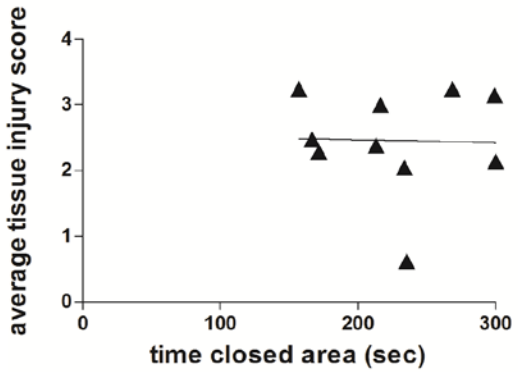

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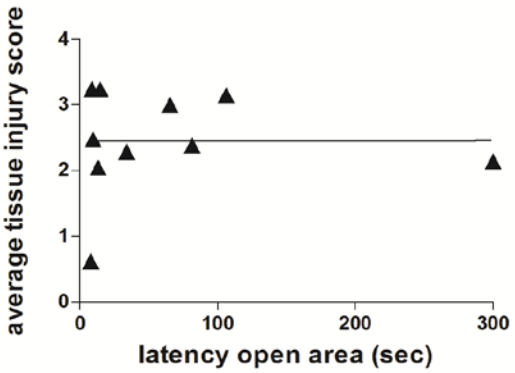


F

Figure 3-6. The correlations between self-injury and innate anxiety-related behaviors in the EPM at the pemoline dose of 75 mg/kg. A) There is no significant correlation ($p>0.05$, $r=0.03696$) between the average duration of oral contact and the time spent in the closed arms. B) There is no significant correlation ($p>0.05$, $r=-0.06897$) between the average duration of oral contact and the latency to enter the open arms. C) There is no significant correlation ($p>0.05$, $r=-0.02299$) between the average injury size and the time spent in the closed arms. D) The was no significant correlation ($p>0.05$, $r=-0.1845$) between the average injury size and the latency to enter the open arms. E) There is no significant correlation ($p>0.05$, $r= 0.07927$) between the average tissue injury score and the time spent in the closed arms. F) There was no significant correlation ($p>0.05$, $r=-0.006098$) between the average tissue injury score and the latency to enter the open arms. One rat in the low dose group (pemoline 75mg/kg) was excluded from analyses with the duration of oral contact due to data loss, which left 9 rats in the group.

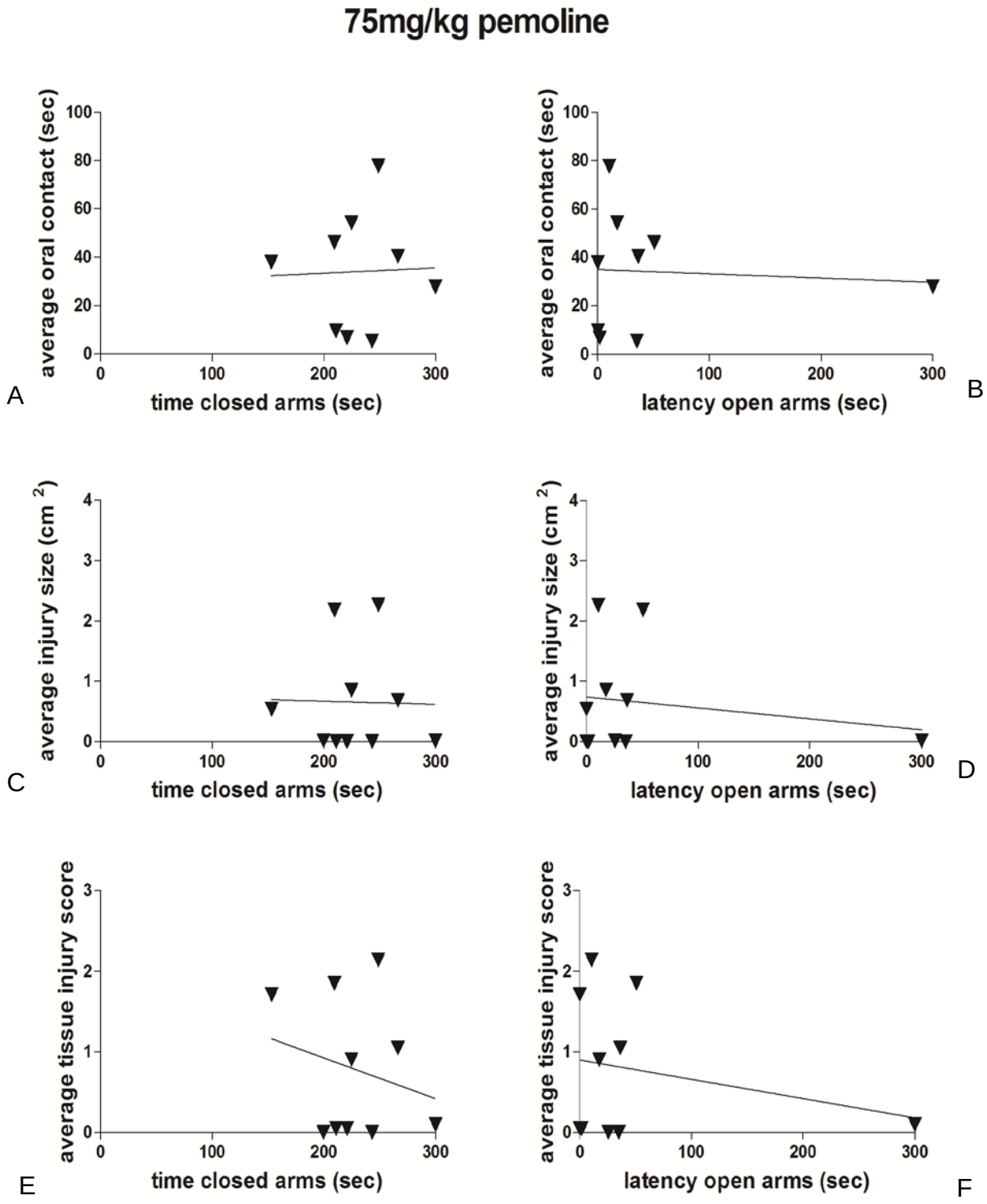
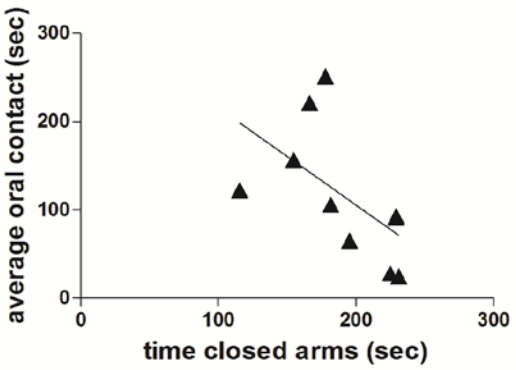
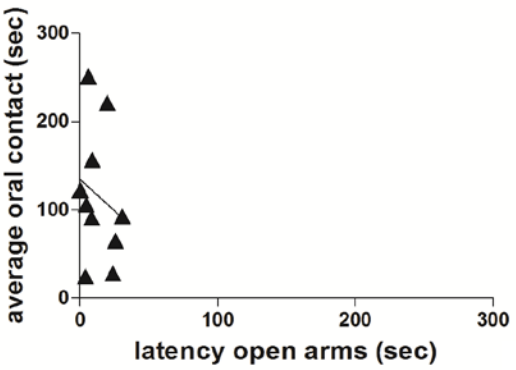

75mg/kg pemoline
average oral contact (sec)
time closed arms (sec)
A
average oral contact (sec)
latency open arms (sec)
B
average injury size (cm 2)
time closed arms (sec)
C
average injury size (cm 2)
latency open arms (sec)
D
average tissue injury score
time closed arms (sec)
E
average tissue injury score
latency open arms (sec)
F

Figure 3-7. The correlations between self-injury and innate anxiety-related behaviors in the EPM at the pemoline dose of 100 mg/kg. A) There is a significant negative correlation ($p<0.05$, r=-0.5695) between the average duration of oral contact and the time spent in the closed arms. B) There is no significant correlation ($p>0.05$, r=-0.2034) between the average duration of oral contact and the latency to enter the open arms. C) There is a significant negative correlation ($p<0.01$, r=-0.7522) between the average injury size and the time spent in the closed arms. D) The was no significant correlation ($p>0.05$, r=-0.3406) between the average injury size and the latency to enter the open arms. E) There is no significant correlation ($p>0.05$, r= -0.462) between the average tissue injury score and the time spent in the closed arms. F) There was no significant correlation ($p>0.05$, r=0.2857) between the average tissue injury score and the latency to enter the open arms.

100 mg/kg pemoline



A



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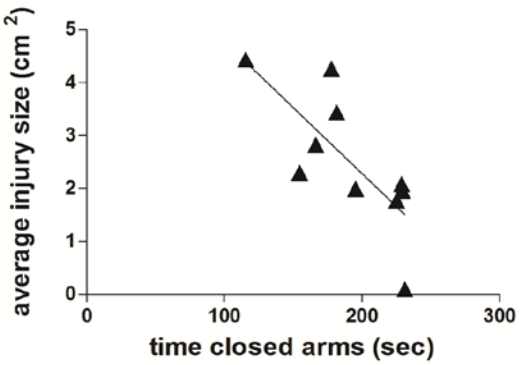
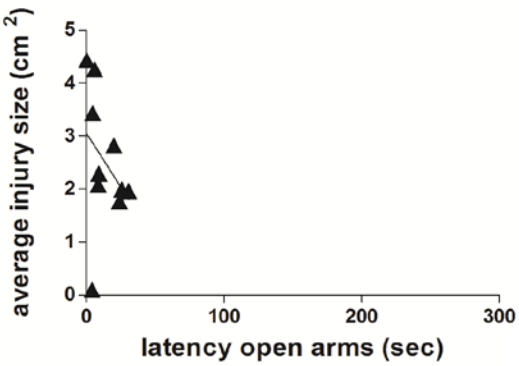
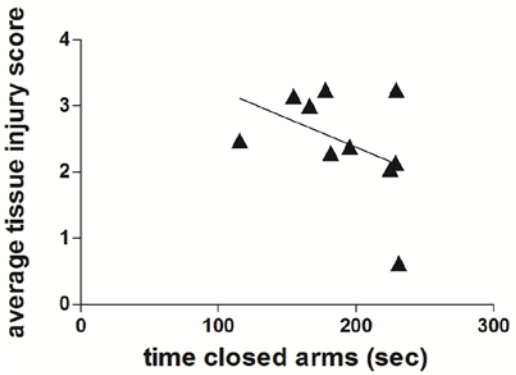


C



D



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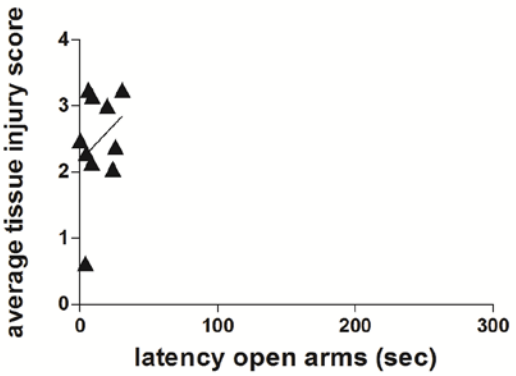
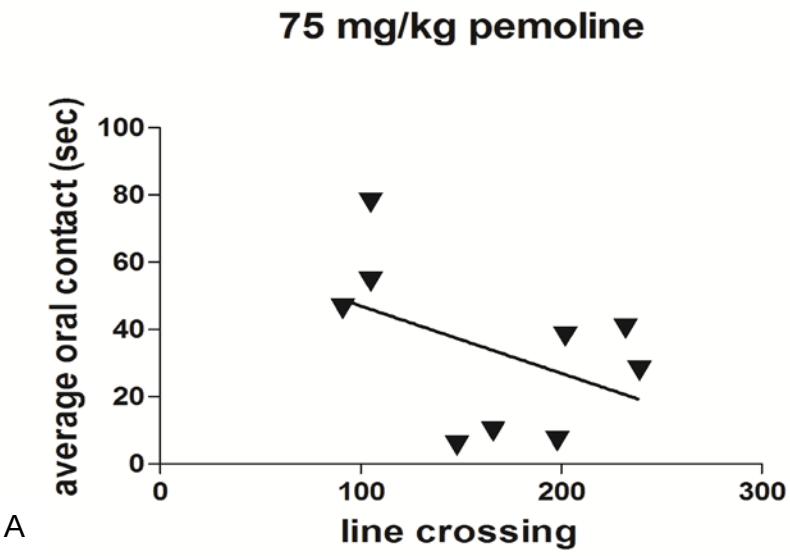
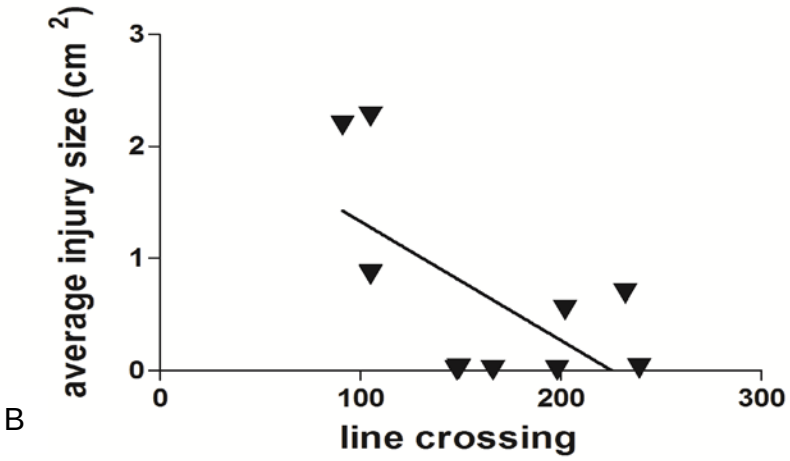


F

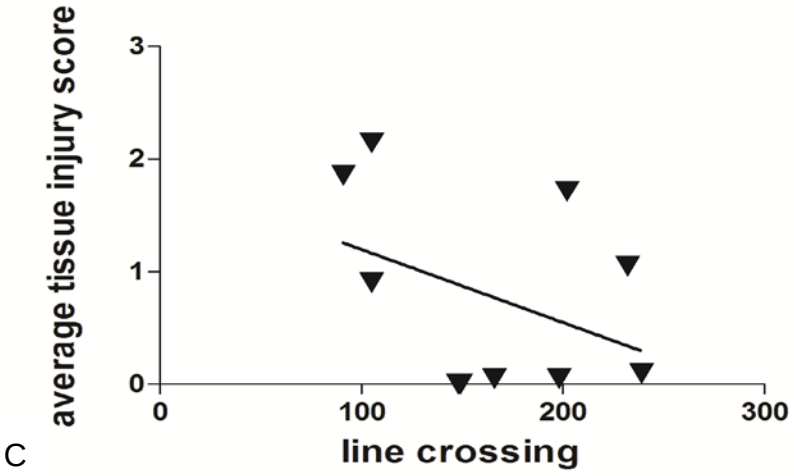
Figure 3-8. The correlations between self-injury and locomotor response to mild stress in CCT at the pemoline dose of 75 mg/kg. A) There was no significant correlation ($p>0.05$, $r=-0.4643$) between the average oral contact and the counts of line crossing. B) There was a significant negative correlation ($p<0.05$, $r=-0.6382$) between the average injury size and the counts of line crossing. C) There was no significant correlation ($p>0.05$, $r=-0.2141$) between the average tissue injury score and the counts of line crossing.


75 mg/kg pemoline
average oral contact (sec)
100
80
60
40
20
0
0
100
200
300
line crossing

A

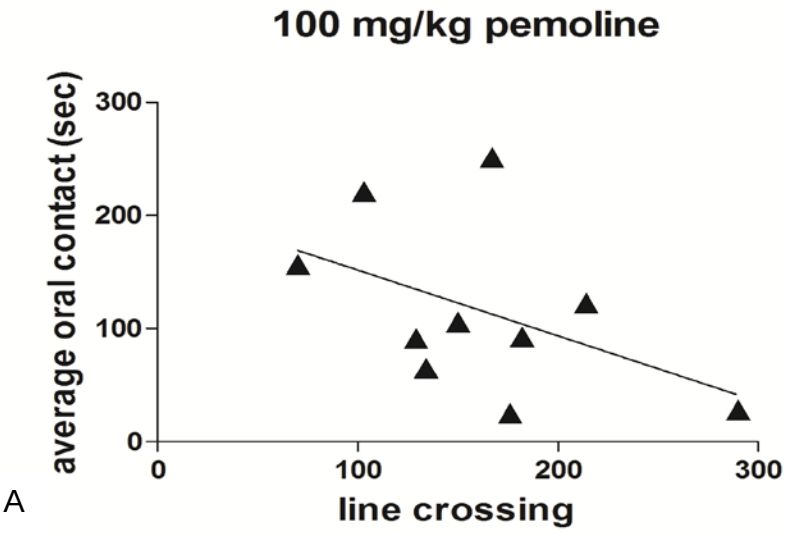

average injury size (cm 2)
3
2
1
0
0
100
200
300
line crossing

B

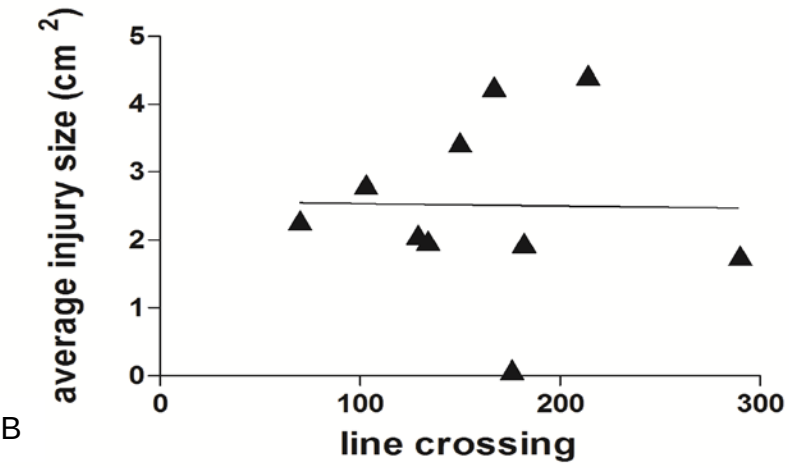

average tissue injury score
3
2
1
0
0
100
200
300
line crossing

C

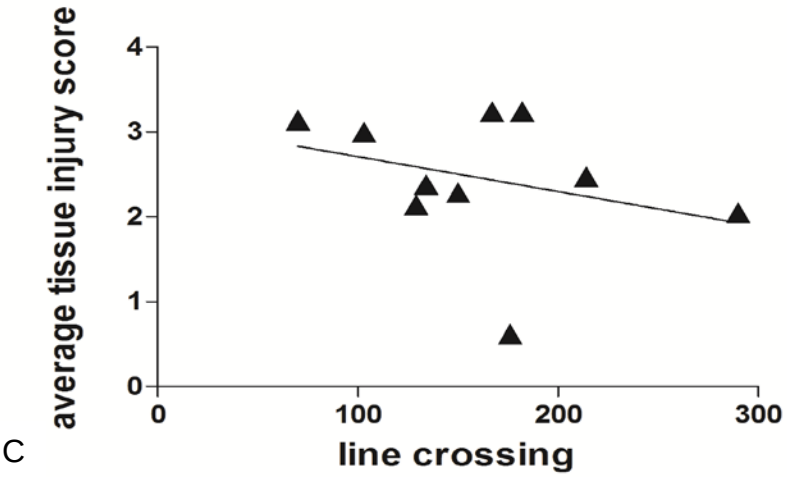
Figure 3-9. The correlations between self-injury and locomotor response to mild stress in CCT at the pemoline dose of 100 mg/kg. A) There was no significant correlation ($p>0.05$, $r=-0.4725$) between the average oral contact and the counts of line crossing. B) There was no significant correlation ($p>0.05$, $r=-0.01626$) between the average injury size and the counts of line crossing. C) There was no significant correlation ($p>0.05$, $r=-0.2128$) between the average tissue injury score and the counts of line crossing.


100 mg/kg pemoline
average oral contact (sec)
300
200
100
0
0
100
200
300
line crossing

A


average injury size (cm 2)
5
4
3
2
1
0
0
100
200
300
line crossing

B


average tissue injury score
4
3
2
1
0
0
100
200
300
line crossing

C

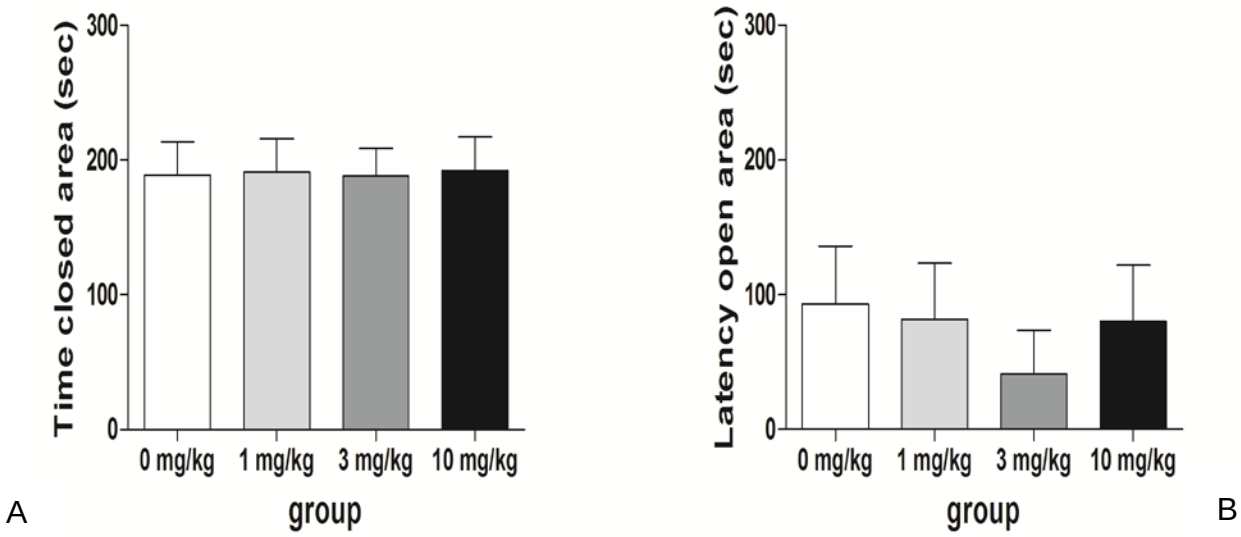


Figure 3-10. Evaluation of balanced group assignments for Experiment 2. The balance of the group assignments was confirmed as the means of A) time spent in the closed area in the OFT and B) latency to enter the closed area in the OFT were not significantly different across all groups with different FG7142 doses (n=9, for each dose and the vehicle).

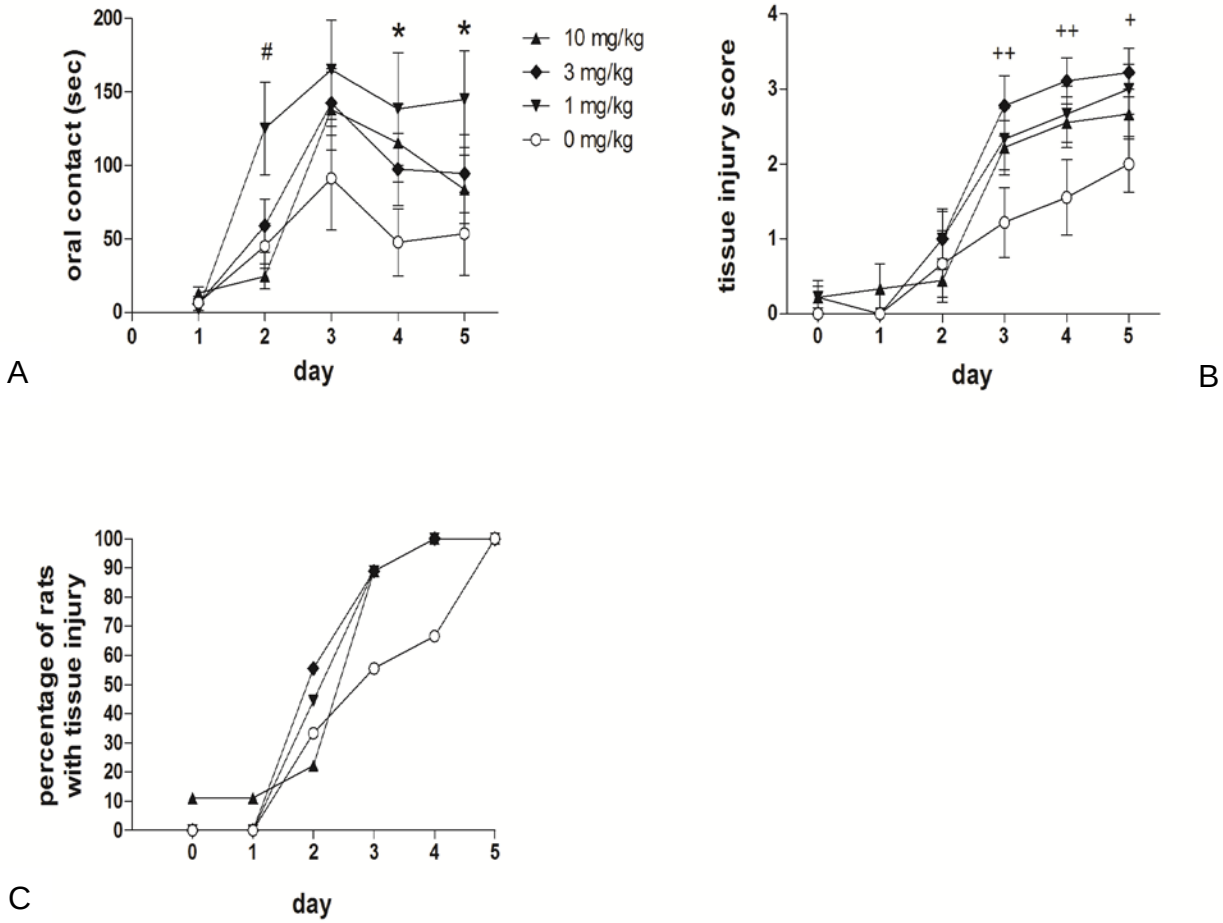


Figure 3-11.FG7142-induced elevation in expression of self-injury. A) Duration of oral contact; B) tissue injury score, C) percentage of rats with tissue injury. Significant differences among the different FG7142 doses and the vehicle are presented as follow: +$p<0.05$ and ++$p<0.01$ for comparisons between FG7142 3mg/kg and vehicle; *$p<0.05$ for comparisons between FG7142 1 mg/kg and vehicle; #$p<0.05$ for comparisons between FG7142 10mg/kg and 1 mg/kg. The data on day 5 of two rats in the vehicle groups were replaced by the corresponding data of day 4, because one rat was injected at the dose of FG7142 1mg/kg and the other rat was injected at the dose of FG7142 3mg/kg by mistake both at the morning of day 5.

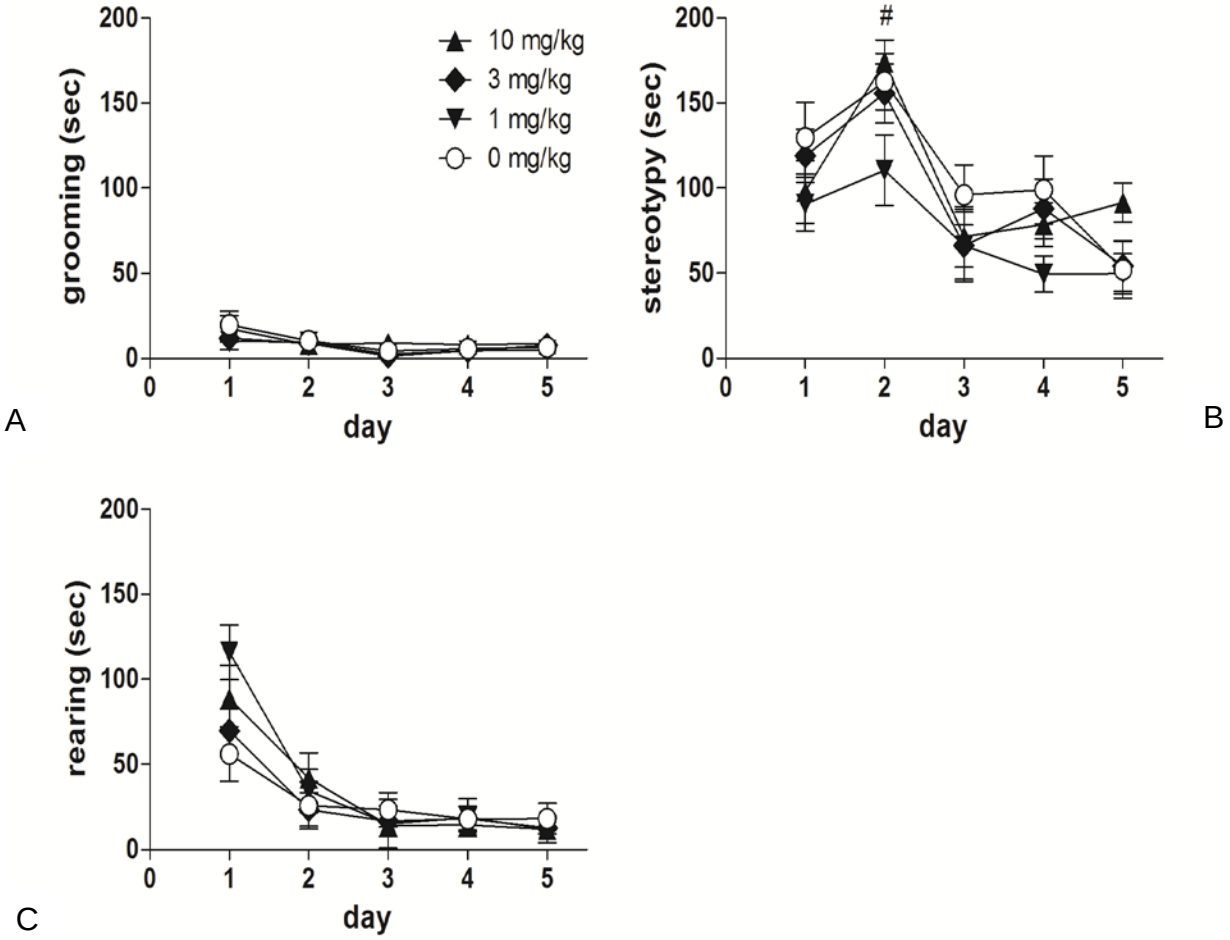


Figure 3-12. Between-group differences in duration of ancillary behaviors in experiment 2. A) Duration of grooming, B) duration of stereotypy; C) duration of rearing. The significant difference between the group of FG7142 10 mg/kg and the group of FG7142 1mg/kg is presented as #P<0.05. The data on day 5 of two rats in the vehicle groups were replaced by the corresponding data of day 4, because one rat was injected at the dose of FG7142 1mg/kg and the other rat was injected at the dose of FG7142 3mg/kg by mistake on the morning of day 5.

CHAPTER 4
DISCUSSION

Dose Effects of Pemoline on Self-Injury

The expression of self-injury among different pemoline doses was dose-orderly for all four dependent measures (percentage of rats that self-injured, self-injurious oral contact, injury size, and tissue injury scores), which was in concordance with a previous finding (Kies and Devine, 2004). It is not clear why the magnitude of the expression of self-injury induced by the 100 mg/kg/day dose was larger in the current study than it was in the previous study, although the strain differences and the improved method for getting pemoline into suspension might contribute to this discrepancy. The 100 mg/kg/day dose of pemoline caused moderate to severe self-injury in all the rats (except 1 rat only exhibited very mild self-injury) of the group with nearly half of the rats euthanized before completion of the treatment, whereas the 75 mg/kg/day dose produced mild to moderate self-injury in 6/10 rats of the group. It appears that the low dose (75 mg/kg pemoline) is a more appropriate dose for studying individual differences in SIB vulnerability, as the high dose might be too powerful to allow the individual differences in SIB vulnerability to be expressed. Thus, the low dose (75 mg/kg pemoline) was used in the experiment 2 studying the effects of FG7142.

Relations between Innate Anxiety-Related Behaviors and Self-Injury

Since positive correlations were consistently detected between the measures of anxiety related behavior in the OFT, and the self-injury measures in the rats treated with the low dose of pemoline, it appears that higher levels of innate anxiety-related behaviors were correlated with increased vulnerability for pemoline-induced SIB. Although one small negative correlation between average injury size and time spent in

the closed area was detected at the high dose, there were no consistent outcomes found between measures of self-injury and indices of anxiety-related behaviors in OFT at this dose. One possible explanation for the inconsistency in results of the high dose group could be that, the reliability in terms of revealing individual differences in SIB etiology was impaired by the severity of the self-injury produced by the high dose of pemoline.

In order to obtain a stable measure of the innate anxiety-related behaviors in rats, both the EPM and OFT were conducted in Experiment 1. However, there were discrepancies between the two tests of anxiety-related behavior (EPM and OFT) in terms of the pattern of correlations between anxiety-related behaviors and vulnerability for pemoline-induced self-injury. One possible explanation is that there are intrinsic differences in the procedures and the design of the apparatuses. As the open arms of the EPM were 90 cm high above the floor, and the open area of the OFT was at floor level, exploration of the open arms of the EPM may have constituted a greater challenge than exploration of the open area of the OFT did. In addition, as the EPM test session started right away when each individual rat was placed onto the maze, the stress of being handled by the experimenter might have a substantial impact on how the rat behaves at the beginning of the test. In contrast, the OFT started after 1-minute habituation in the start-box (which is also different from OFT procedures that have been reported in other publications). This potentially decreased the handling effects on the results of the OFT. Furthermore, there was no prior handling before the EPM whereas the OFT was conducted after the rats experienced handling during the EPM. This again might make the latter test less stressful than the former one. It has been reported that

Wistar rats exhibit elevated expression of anxiety-related behaviors compared to Long-Evans rats (Shaw et al., 2009). Although there is no direct evidence, the Wistar rats that were used in this study could be particularly sensitive to the stress of the EPM, obscuring the individual differences in the individual rats' behaviors. Moreover, it has been reported that the EPM and OFT may measure different aspects of emotionality. In one study, the anxiolytic drug NKP608 (a NK1 receptor antagonist) exerted anxiolytic-like effects on the OFT but not on the EPM in male spontaneously hypertensive rats（SHR). Conversely, it had a partial anxiolytic-like effect on the EPM but not OFT in male Lewis (LEW) rats (Vendruscolo et al., 2003). However, it should be noted that the version of the OFT used in the current study was different from that used in these other experiments.

Relations between Locomotor Response to Mild Stress and Self-Injury

A previous study using the CCT reported positive correlations between the locomotor counts and self-injury measures (Muehlmann et al., 2011). These correlations were not found in the current study, and there was one negative correlation between the average injury size and the locomotors counts. However, there is evidence that the positive correlations detected in the previous study were driven by highly-responsive rats with high locomotor counts. The locomotor counts in the previous study ranged from 156 to 448 (mean score = 231.50), whereas the locomotor counts in the present study ranged from 70 to 290 (mean score = 160.03). This probably contributed to discrepancies between the two studies. In addition, strain differences (Wistar vs. Long-Evans) of rats used in the two studies might also contribute to the discrepancies in results.

FG7142-Induced Elevation in Expression and Severity of Self-Injury

Since the FG7142-treated rats exhibited greater duration of oral contact, larger tissue injury scores, and a greater percentage of these rats engaged in self-injury (current results) and since FG7142 increases the expression of anxiety-related behaviors in a variety of behavioral tests (Atack et al., 2005; Bueno et al., 2005; Dawson et al., 2006; Hackler et al., 2007; Sena et al., 2003; Stephens et al., 1987), the outcomes of Experiment 2 further support the hypothesis that anxiety-related behaviors and self-injury are positively correlated, as suggested by the results from Experiment 1.

It is likely that the elevated expression of SIB in the FG7142-treated rats in this study is due to elevated anxiety levels. Because beside its effects on anxiety-related behaviors, the ability of FG7142 (a partial inverse agonist of the $GABA_A$ receptor) to increase anxiety states is supported by several lines of evidence regarding neurochemical and neurophysiological changes induced by FG7142 administration. Many studies found that FG7142 increased expression of *c-fos* protein and mRNA in brain regions involved in anxiety-related-behaviors, including prefrontal cortex (PFC), anterior cingulate cortex (ACC), amygdala, paraventricular thalamic nucleus of hypothalamus (PVN), bed nucleus of stria terminalis (BNST), nucleus accumbens (NAc), and striatum (Kurumaji et al., 2003; Lyss et al., 1999). In terms of potential neurophysiological effects, FG7142 augmented the elevation of plasma corticosterone induced by the OFT (Fernandez et al., 2004). Also, FG7142 (20 mg/kg, i.p.) increased extracellular corticosterone in the basolateral amygdala complex of male Wistar rats (Bouchez et al., 2012), which is a brain region highly involved in anxiety. However, changes in mRNA of corticotropin-releasing factor (CRF, a neuropeptide regulating corticosterone level and increasing anxiety-related behaviors) were not detected after

administration of FG7142 (10mg/kg) in male Wistar rats (Funk et al., 2006) and C57 BL/6J mice (Funk et al., 2009) among many brain regions related to anxiety. Future work assessing changes in corticosterone, CRF and their receptors in relation to self-injury mechanisms might be helpful to provide further explanations on the FG7142-induced elevation of self-injury.

For more than 20 years, the foremost neurobiological explanation for SIB was that it is driven by D1 dopamine receptor-mediated supersensitivity of striatal medium spiny neurons (MSNs) (Breese et al., 1985), suggesting the involvement of dopamine neurotransmission. However, contributions of dopaminergic and non-dopaminergic neurotransmission in other parts of the brain have not been studied as closely. Indeed, FG7142-induced changes in dopamine neurotransmission were found within many brain regions involved in anxiety. Increased extracellular dopamine release in medial prefrontal cortex (mPFC) but not in striatum was observed after administration of FG7142 (15 mg/kg i.p.), as assessed by in vivo microdialysis (Moghaddam et al., 1990). Increased concentration of 3, 4-dihydroxyphenylacetic acid (DOPAC, a dopamine metabolite) was also found in the medial prefrontal cortex in rats after FG7142 administration (15 mg/kg i.p.) (Attack et al., 2005). In addition, increased extracellular concentration of homovanillic acid (HVA, another metabolite of dopamine) in NAc was detected starting from day 3 of a sub-chronic FG7142 treatment and this measure peaked at day 5 (Brose et al., 1987) whereas these effects were not found in striatum. FG7142 also increased tyrosine hydroxylase activity in PFC and ventral tegmental area *in vivo* but not in caudate-putamen or substantia nigra, whereas it decreased tyrosine hydroxylase activity in striatum *in vitro* (Knorr et al., 1989). Thus it is possible that

interactions between FG7142 and dopamine neurotransmission within brain regions related to anxiety contributed to the observed adverse effects of FG7142 on self-injury in the current study.

Intriguingly, FG7142-induced increase in dopamine turnover was blocked by the glycine/NMDA antagonist (+) HA966 (Murphy et al., 1996), suggesting the involvement of glutamate neurotransmission. Glutamate neurotransmission is also implicated in pemoline-induced self-injury (King, Au, and Poland, 1995; Muehlmann and Devine, 2008), as the NMDA antagonist MK801 decreased self-injury in the pemoline model. This line of evidence suggests a potential overlap between FG7142-induced anxiety and pemoline-induced self-injury, which might explain the augmentation of pemoline-induced self-injury during repeated FG7142 treatment.

As reviewed by Etkin et al. (2011), the prefrontal cortex is likely to exert a modulatory function on anxiety-related behaviors by regulating neuronal activities within amygdala. mPFC inputs decreased the possibility of firing in neurons of basolateral complex of amygdala (BLA), possibly by activating inhibitory interneurons within this brain region. This top-down suppression from mPFC to amygdala was disrupted by excessive-stimulation on PFC dopamine receptors (Rosenkranz et al., 2001). It is possible that FG7142 induced changes in dopamine neurotransmission would also impair the PFC control over amygdala. This needs to be tested with further experiments. Despite the prominent role of the amygdala in anxiety, the role of this region in self-injury is not clear. However, a case study reported that deep brain stimulation in basolateral amygdala improved SIB in an autistic boy (Sturm et al., 2012). Nevertheless, this result should be interpreted cautiously because it is a single case,

uncontrolled design, and the results were based on subjective scores documented by his parents who didn't received psychiatric, psychological or medical training.

Beside the associations between FG7142 and dopamine neurotransmission, there is also evidence suggesting involvement of serotonin neurotransmission in FG7142-induced elevation of anxiety. For example, pretreatment with the selective 5-HT_{2C} receptor antagonist SB 242084 blocked FG7142 induced increases in anxiety-related behaviors and the increase in blood-oxygen-level-dependent (BOLD) signal in amygdala, hypothalamus and hippocampus (Hackler et al., 2007). However, the role of serotonin in self-injury remains controversial. Fluoxetine, a selective serotonin reuptake inhibitor (SSRI) attenuated SIB (Fontenot et al., 2005; Fontenot et al., 2009) in non-human primates. Pretreatment with another SSRI, paroxetine significantly increased the severity of SIB (Turner et al., 1999) in the pemoline model in rats. Based on current knowledge, it is possible that serotonin neurotransmission is involved in anxiety-related behaviors augmented by FG7142 whereas it is not clear if it has a role in FG7142-induced elevation in self-injury.

Regarding all evidence discussed above about changes in neurotransmission within brain regions involved in anxiety, one possibility is that FG7142-induced increases in self-injury might be due to certain overlaps between the neurochemical mechanisms underlying anxiety and self-injury. However, more effort needs to be expended on elucidating the underlying neurochemical mechanisms of SIB, especially for the role of dopamine, serotonin and glutamate, as well as the neuroanatomical structures that are involved, before we can further confirm (or reject) this possibility.

Implications for Neurodevelopmental Disorders

Although the comorbidity between anxiety and self-injury is not well-established, anxiety disorders have been reported among self-injurious patients with neurodevelopmental disorders. According to a meta-analysis study, about 40% of young people with autistic spectrum disorders (ASD) meet diagnostic criteria for at least one DSM IV anxiety disorder (Van Steensel et al., 2011). Since approximately 50% of people with ASD exhibit SIB (Richards et al., 2012), there is a potential for significant overlap between these subsets of autistic individuals. In the case of fragile X syndrome (FXS), the proportion of SIB patients has been estimated at about 55% (Richards et al., 2012). And there is a significantly higher ratio of co-occurring anxiety found in those individuals with FXS who self-injured compared with those who did not self-injure (Symons et al., 2010). As for Lesch-Nyhan disease (LND) for which self-injury is its hallmark, higher anxious/depressed level was observed in LND patients compared to both healthy controls and patients with Lesch–Nyhan variants (LNV) who did not self-injure (Schretlen et al., 2005). These findings converge to suggest a potential overlap between pathological mechanisms underlying anxiety and self-injury, yet no conclusion can be reached without further information regarding the specific comorbidity between anxiety and self-injury among the above mentioned neurodevelopmental disorders. If there actually are correlations between anxiety and self-injury in defined clinical populations, causal relations still cannot be easily determined unless we can explain more fully the underlying neurobiological/neuropathological mechanisms.

Conclusion

This study provided evidence for the role of anxiety in self-injury induced by pemoline in a rat model, by demonstrating the positive correlations between innate

anxiety-related behaviors and self-injury, and the adverse effect of an anxiogenic agent FG7142 on self-injury. Future studies assessing anxiety-related behavioral/hormonal changes before, during and after the pemoline treatment and investigating neurochemical changes underlying FG7142-induced augmentation of self-injury will be helpful in order to facilitate a greater understanding of the role of anxiety in self-injury.

LIST OF REFERENCES

Atack JR, Bayley PJ, Seabrook GR, Wafford KA, McKernan RM, Dawson GR (2006). L-655,708 enhances cognition in rats but is not proconvulsant at a dose selective for alpha5-containing GABAA receptors. *Neuropharmacology* **51:**1023-1029.

Bouchez G, Millan MJ, Rivet JM, Billiras R, Boulanger R, Gobert A (2012). Quantification of extracellular levels of corticosterone in the basolateral amygdaloid complex of freely-moving rats: a dialysis study of circadian variation and stress-induced modulation. *Brain Res* **1452:** 47-60.

Brahm NC, Fast GA, Brown, RC (2008). Buspirone for autistic disorder in a woman with an intellectual disability. *Ann. Pharmacother* **42:** 131-137.

Breese GR, Baumeister A, Napier TC, Frye GD, Mueller RA (1985). Evidence that D-1 dopamine receptors contribute to the supersensitive behavioral responses induced by L-dihydroxyphenylalanine in rats treated neonatally with 6-hydroxydopamine. *J Pharmacol Exp Ther* **235:** 287-95.

Brose N, O'Neill RD, Boutelle MG, Anderson SM, Fillenz M (1987). Effects of an anxiogenic benzodiazepine receptor ligand on motor activity and dopamine release in nucleus accumbens and striatum in the rat. *J Neurosci* **7:** 2917-26.

Bueno CH, Zangrossi H, Jr., Nogueira RL, Soares VP, Viana MB (2005). Panicolytic-like effect induced by the stimulation of GABAA and GABAB receptors in the dorsal periaqueductal grey of rats. *Eur J Pharmacol* **516:**239-246.

Dawson GR, Maubach KA, Collinson N, Cobain M, Everitt BJ, Macleod AM, *et al* (2006). An inverse agonist selective for alpha 5 subunit-containing GABAA receptors enhances cognition. *J Pharmacol Exp Ther* **316:**1335-1345.

Di Pierro R, Sarno I, Perego S, Gallucci M, Madeddu F (2012). Adolescent nonsuicidal self-injury: the effects of personality traits, family relationships and maltreatment on the presence and severity of behaviours. *Eur Child Adolesc Psychiatry* **21:**511-20.

Esposito-Smythers C, Goldstein T, Birmaher B, Goldstein B, Hunt J, Ryan N *et al* (2010). Clinical and psychosocial correlates of non-suicidal self-injury within a sample of children and adolescents with bipolar disorder. *J Affect Disord* **125:**89-97.

Ernst M, Zametkin AJ, Matochik JA, Pascualvaca D, Jons PH, Hardy K *et al* (1996). Presynaptic dopaminergic deficits in Lesch-Nyhan disease. *N Engl J Med* **334:**1568-72.

Etkin A, Egner T, Kalisch R (2011). Emotional processing in anterior cingulate and medial prefrontal cortex. *Trends Cogn Sci* **15:**85-93.

Evans AK, Lowry CA (2007). Pharmacology of the beta-carboline FG-7,142, a partial inverse agonist at the benzodiazepine allosteric site of the GABA A receptor: neurochemical, neurophysiological, and behavioral effects. *CNS Drug Reviews* **13:**475-501

Fernandez F, Misilmeri MA, Felger JC, Devine DP (2004). Nociceptin/orphanin FQ increases anxiety-related behavior and circulating levels of corticosterone during neophobic tests of anxiety. *Neuropsychopharmacology* **29:**59-71.

Fontenot MB, Musso MW, McFatter RM, Anderson GM (2009). Dose-finding study of fluoxetine and venlafaxine for the treatment of self-injurious and stereotypic behavior in rhesus macaques (Macaca mulatta). *J Am Assoc Lab Anim Sci* **48:**176-184.

Fontenot MB, Padgett EE 3rd, Dupuy AM, Lynch CR, De Petrillo PB, Higley JD (2005). The effects of fluoxetine and buspirone on self-injurious and stereotypic behavior in adult male rhesus macaques. *Comp Med* **55:** 67-74.

Funk D, Li Z, Lê AD (2006). Effects of environmental and pharmacological stressors on c-fos and corticotropin-releasing factor mRNA in rat brain: Relationship to the reinstatement of alcohol seeking. *Neuroscience* **138:**235-43.

Funk D, Li Z, Coen K, Lê AD (2008). Effects of pharmacological stressors on c-fos and CRF mRNA in mouse brain: relationship to alcohol seeking. *Neurosci Lett* **444:**254-8.

Hackler EA, Turner GH, Gresch PJ, Sengupta S, Deutch AY, Avison MJ *et al* (2007). 5-Hydroxytryptamine2c receptor contribution to m-chlorophenylpiperazine and N-methyl-beta-carboline-3-carboxamide-induced anxiety-like behavior and limbic brain activation. *J Pharmacol Exp Ther* **320:**1023-1029.

Hallett V, Lecavalier L, Sukhodolsky DG, Cipriano N, Aman MG, McCracken JT *et al* (2013). Exploring the Manifestations of Anxiety in Children with Autism Spectrum Disorders. *J Autism Dev Disord;* e-pub ahead of print 12 February 2013. doi: 10.1007/s10803-013-1775-1

Kies SD, Devine DP (2004). Self-injurious behaviour: a comparison of caffeine and pemoline models in rats. *Pharmacol Biochem Behav* **79:** 587-598.

King BH, Au D, Poland RE (1995). Pretreatment with MK-801 inhibits pemoline-induced self-biting behavior in prepubertal rats*. Dev Neurosci* **17:**47-52.

Knorr AM, Deutch AY, Roth RH (1989). The anxiogenic beta-carboline FG-7142 increases in vivo and in vitro tyrosine hydroxylation in the prefrontal cortex. *Brain Res* **495:**355-61.

Kurumaji A, Umino A, Tanami M, Ito A, Asakawa M, Nishikawa T (2003). Distribution of anxiogenic-induced c-Fos in the forebrain regions of developing rats. *J Neural Transm* **110:**1161-8.

Lyss PJ, Andersen SL, LeBlanc CJ, Teicher MH (1999). Degree of neuronal activation following FG-7142 changes across regions during development. *Brain Res Dev Brain Res* **116:**201-3.

Major CA, Kelly BJ, Novak MA, Davenport MD, Stonemetz KM, Meyer JS (2009). The anxiogenic drug FG7142 increases self-injurious behavior in male rhesus monkeys (Macaca mulatta). *Life Sci* **85:** 753-758.

Moghaddam B, Roth RH, Bunney BS (1990). Characterization of dopamine release in the rat medial prefrontal cortex as assessed by in vivo microdialysis: comparison to the striatum. *Neuroscience* **36:**669-76.

Muehlmann AM, Devine DP (2008). Self-injurious behavior: Individual differences in neurotransmitter concentrations using an animal model. Keystone Symposium: Towards Identifying the Pathophysiology of Autistic Syndromes.

Muehlmann AM, Wilkinson JA, Devine DP (2011). Individual differences in vulnerability for self-injurious behavior: studies using an animal model. *Behav Brain Res* **217:** 148-154.

Mueller K, Nyhan WL (1982). Pharmacologic control of pemoline induced self-injurious behavior in rats. *Pharmacol Biochem Behav* **16:**957-63.

Murphy BL, Arnsten AF, Jentsch JD, Roth RH (1996). Dopamine and spatial working memory in rats and monkeys: pharmacological reversal of stress-induced impairment. *J Neurosci* **16:**7768-75.

Peça J, Feliciano C, Ting JT, Wang W, Wells MF, Venkatraman TN *et al* (2011). Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. *Nature* **472:**437-42.

Richards C, Oliver C, Nelson L, Moss J (2012). Self-injurious behaviour in individuals with autism spectrum disorder and intellectual disability *J Intellect Disabil Res* **56:**476-89.

Rosenkranz JA, Grace AA (2001). Dopamine attenuates prefrontal cortical suppression of sensory inputs to the basolateral amygdala of rats. *J Neurosci* **21:**4090-103.

Schretlen DJ, Ward J, Meyer SM, Yun J, Puig JG, Nyhan WL *et al* (2005). Behavioral aspects of Lesch-Nyhan disease and its variants*. Dev Med Child Neurol* **47:**673-7.

Sena LM, Bueno C, Pobbe RL, Andrade TG, Zangrossi H, Jr., Viana MB (2003). The dorsal raphe nucleus exerts opposed control on generalized anxiety and panic-related defensive responses in rats. *Behav Brain Res* **142:**125-133.

Shaw FZ, Chuang SH, Shieh KR, Wang YJ (2009). Depression- and anxiety-like behaviors of a rat model with absence epileptic discharges. *Neuroscience* **160:** 382-393.

Shmelkov SV, Hormigo A, Jing D, Proenca CC, Bath KG, Milde T *et al* (2010). Slitrk5 deficiency impairs corticostriatal circuitry and leads to obsessive-compulsive-like behaviors in mice. *Nat Med* **16:**598-602, 1p following 602.

Stephens DN, Schneider HH, Kehr W, Jensen LH, Petersen E, Honore T (1987). Modulation of anxiety by betacarbolines and other benzodiazepine receptor ligands: Relationship of pharmacological to biochemical measures of efficacy. *Brain Res Bull* **19:**309-318.

Sturm V, Fricke O, Bührle CP, Lenartz D, Maarouf M, Treuer H *et al* (2013). DBS in the basolateral amygdala improves symptoms of autism and related self-injurious behavior: a case report and hypothesis on the pathogenesis of the disorder. *Front Hum Neurosci* **6:**341.

Symons FJ, Byiers BJ, Raspa M, Bishop E, Bailey DB (2010). Self-injurious behavior and fragile X syndrome: findings from the national fragile X survey. Am. J. Intellect. *Dev Disabil* **115:** 473-481.

Tsiouris JA, Cohen IL, Patti PJ, Korosh WM (2003). Treatment of previously undiagnosed psychiatric disorders in persons with developmental disabilities decreased or eliminated self-injurious behavior. *J Clin Psychiatry* **64:**1081-1090.

Turner C, Panksepp J, Bekkedal M, Borkowski C, Burgdorf J (1999). Paradoxical effects of serotonin and opioids in pemoline-induced self-injurious behavior. *Pharmacol Biochem Behav* **63:**361-6.

van Steensel FJ, Bögels SM, Perrin S (2011). Anxiety disorders in children and adolescents with autistic spectrum disorders: a meta-analysis. *Clin Child Fam Psychol Rev* **14:**302-17.

Vendruscolo LF, Takahashi RN, Brüske GR, Ramos A (2003). Evaluation of the anxiolytic-like effect of NKP608, a NK1-receptor antagonist, in two rat strains that differ in anxiety-related behaviors. *Psychopharmacology (Berl)* **170:**287-93.

Welch JM, Lu J, Rodriguiz RM, Trotta NC, Peca J, Ding JD *et al* (2007). Cortico-striatal synaptic defects and OCD-like behaviours in Sapap3-mutant mice. *Nature* **448:**894-900.

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